
**Soil quality — Sampling of soil
invertebrates —**

Part 4:
**Sampling, extraction and
identification of soil-inhabiting
nematodes**

Qualité du sol — Prélèvement des invertébrés du sol —

*Partie 4: Prélèvement, extraction et identification des nématodes du
sol*





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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 23611-4:2007), which has been technically revised. The main changes are as follows:

- examples of the use of nematodes in soil monitoring programmes have been added (including presentation of their results) as an informative annex (see [Annex D](#)).

A list of all parts in the ISO 23611 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document has been drawn up since there is a growing need for the standardization of terrestrial zoological field methods. Such methods, mainly covering the sampling, extraction and handling of soil invertebrates, are necessary for the following purposes:

- biological classification of soils including soil quality assessment [37],[42],[57];
- terrestrial bio-indication and long-term monitoring [25],[28],[31],[50];
- evaluation of the effects of chemicals on soil animals in the field (see ISO 11268-3[4]).

Data for these purposes are gained by standardized methods since they can form the basis for far-reaching decisions (e.g. whether a given site should be remediated or not). In fact, the lack of such standardized methods is one of the most important reasons why bio-classification and bio-assessment in terrestrial (i.e. soil) habitats has so far been relatively rarely used in comparison to aquatic sites.

Nematodes are an important and major part of the soil fauna. Some authors estimate that this group is probably the most dominant one of the multicellular organisms (Metazoa) on earth[52]. Nematodes occur from the Antarctic to the tropics and from deep sea sediments to mountain regions. They are active in every place with sufficient water and organic material. The species diversity and functional variety are impressive[14]. Nematodes are commonly known as parasites of animals and plants, but the major part of the nematode fauna participates in decomposition processes by feeding on bacteria and fungi.

Nematodes occur in high numbers ($0,2 \times 10^6 \text{ m}^{-2}$ to $9 \times 10^6 \text{ m}^{-2}$) and with a high (10 to 100 species) diversity in almost every soil sample[12]. Moreover, there is a broad ecological spectrum of feeding types and food web relations among the nematodes such as bacterivores, fungivores, herbivores, predators and omnivores[57],[58]. These factors make the group highly suitable as indicators for ecological soil quality[56], but standardization of methods is urgently needed for comparison and combination of results.

In the past 100 years, nematology has developed strongly from the viewpoint of agriculture, advisory sampling and phytosanitary regulations because some terrestrial nematodes cause a lot of damage in crops. With respect to methods, there are several “schools” in different parts of the world with their own history, practical advantages and disadvantages. A comprehensive overview is given by Oostenbrink[14] and Southey[48],[49]. The more recently described methods (or variants) are often developed with special interest to certain plant parasitic species. Within the past 20 years new methods have evolved that allow a DNA-based taxonomic identification of nematode species[21],[34],[54]. This opens the taxonomic analysis of nematodes to a broader community of non-specialists.

Since Bongers[16] introduced the Maturity Index, the use of nematodes in bio-indication for soil quality has increased rapidly[56]. Nematodes are now used for ecological soil research and monitoring in several countries all over the world. Monitoring activities make special demands on methodology, for instance, that a large number of soil samples is processed on a routine basis against reasonable costs. Some of the methods originally developed for advisory sampling in agriculture are very suitable for ecological research. They form the basis for specific variants described in this document.

The nematodes that are characterized by the proposed procedure are all the free-living forms of nematodes found in soil. They include non-plant-feeding nematodes as well as ectoparasitic plant-feeding nematodes and free-living stage of endoparasitic nematodes. The quantification of obligate plant-feeding nematodes in roots requires specific methods. Basic information on the ecology of nematodes and their use as bio-indicators can be found in the bibliography.

Soil quality — Sampling of soil invertebrates —

Part 4:

Sampling, extraction and identification of soil-inhabiting nematodes

1 Scope

This document specifies a method for sampling and handling free-living nematodes from terrestrial field soils as a prerequisite for using them as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms).

This document applies to all terrestrial biotopes in which nematodes occur. The sampling design of field studies in general is specified in ISO 18400-101.

This document is not applicable to aquatic nematodes because of differences in the sample matrix (e.g. water column). Methods for some other soil organism groups such as earthworms, collembolans, enchytraeids or macro-invertebrates are covered in ISO 23611-1, ISO 23611-2, ISO 23611-3 and ISO 23611-5.

This document does not cover the pedological characterization of the site which is highly recommendable when sampling soil invertebrates. ISO 10390, ISO 10694, ISO 11272, ISO 11274, ISO 11277, ISO 11461 and ISO 11465 include suitable procedures for measuring pH, particle size distribution, C/N ratio, organic carbon content and water-holding capacity.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp/>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

nematode

small, non-segmented free-living worm (up to a few millimetres in length) belonging to the class Nematoda

Note 1 to entry: Nematodes without a soil-inhabiting stage are not included in this context.

3.2

location

study area or plot that is characterized based on the composition of (among others) the nematode fauna